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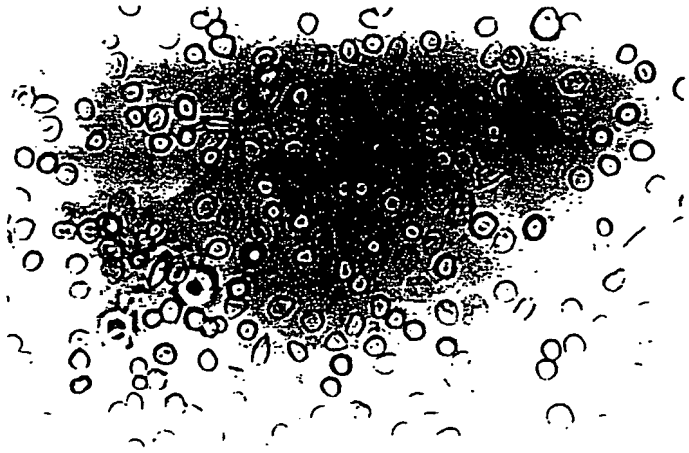
(54) Title: METHODS OF ISOLATION, CRYOPRESERVATION, AND THERAPEUTIC USE OF HUMAN AMNIOTIC EPITHELIAL CELLS



Human amniotic epithelial cells just after isolation from amnion.

(57) Abstract: The present invention is directed to human amniotic epithelial cells derived from placenta at delivery, and the methods for isolating, culturing, and cryopreserving them for future therapeutic uses. Additionally, the present invention is directed to methods for inducing differentiation of these multipotentials cells, for assaying the cell types derived, and for manipulating the cells by gene transfection and other means for therapeutic applications, icluding but not limited to enzyme replacement and gene therapy, tissue regeneration and replacement, as well as burn and wound dressings.

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METHODS OF ISOLATION, CRYOPRESERVATION, AND THERAPEUTIC USE OF HUMAN AMNIOTIC EPITHELIAL CELLS

I. Field of the Invention

This invention relates to the isolation, culture, and preservation by cryogenic techniques of human amniotic epithelial cells and for methods of treatment therefor.

II. Background of the Invention

There is considerable interest in the isolation and culture of pluripotential human embryonic stem cells for the purpose of tissue transplantation, reconstructive surgery, and gene therapy. These stem cells, being undifferentiated, have the potential to develop into any tissue in the body, given the right environment and differentiation factors. Two reports describing the isolation, long-term culture, and differentiation of such cells have generated tremendous excitement in this regard and are herein incorporated by reference (1, 2). However, along with the excitement is a growing apprehension about the ethics of deriving human stem cells from aborted fetuses or human embryos, even in the earliest stage of development. In addition, federal bans on public support for human embryonic research restrict widespread research and development in this area.

The instant invention is directed to the isolation, culture, and preservation by cryogenic techniques of human amniotic epithelial cells which can be used as a source of multipotential cells for tissue regeneration. There is much evidence in the literature that argues for the multipotentiality of such cells. For example, amniotic membrane transplantation has been demonstrated to restore epithelialization of corneal surface in patients with both limbal stem cell deficiency (3) and persistent epithelial defects with

sterile ulceration (4). Amniotic membranes have also been used as dressings following dermal abrasion (5), severe burns (6, 7), chronic leg ulceration (8), and for the treatment of vaginal agenesis and vaginal reconstruction after vaginectomy (9, 10). Clinical arthroplastic studies by Dr. Jonathan Sackier and colleagues further demonstrate that fresh amnion stimulates neochondrogenesis and cartilage reformation in rabbit knee joints denuded of endogenous cartilage (11). These studies collectively suggest that amniotic cells have at least multipotential, if not pluripotential, character in tissue regeneration *in vivo*.

Aside from its uses in reconstructive surgery, amniotic cells have also demonstrated usefulness in the treatment of inborn errors of metabolism, such as Niemann-Pick disease, which involves a genetic deficiency in the lysosomal enzyme sphingomyelinase, and GM_1 – ganglosidosis. For example, Scaggiante and coworkers utilized isolated human amniotic epithelial cells directly to treat Niemann-Pick patients (12), while Sakuragawa et al. transfected amniotic cells with β-galactosidase before transplantation to correct for GM_1 – gangliosidosis (13). Because of the reportedly low expression of histocompatibility antigens on amniotic epithelial cells (14), these cells are ideal candidates for use as carriers in gene therapy. All references cited herein are hereby incorporated by reference.

In addition to having many clinical applications, human amniotic epithelial cells are readily available in human placenta, which is a normally discarded byproduct of a natural birth process. This availability eliminates ethical concerns that have been raised against the use of human embryonic stem cells.

III. Summary of the Invention

The present invention is directed to human amniotic epithelial cells derived from placenta at delivery, and the methods for isolating, culturing, and cryopreserving them for future therapeutic uses. Additionally, the present invention is directed to methods for inducing differentiation of these multipotential cells, for assaying the cell types derived, and for manipulating the cells by gene transfection and other means for therapeutic applications, including but not limited to enzyme replacement and gene therapy, tissue regeneration and replacement, as well as burn and wound dressings.

An object of the present invention is to describe a method for isolating pure human amniotic epithelial cells, free from contaminating fibroblasts.

Another object of the present invention is to describe the culture of human amniotic epithelial cells.

Another object of the present invention is to describe long-term culture of human amniotic epithelial cells on feeder cells matched or unmatched to the same donor as the epithelial cells.

Another object of the present invention is to describe the characterization of human amniotic epithelial cells in terms of cell morphology, epithelial membrane antigen and cytokeratin staining, and gap junctional communication.

Another object of the present invention is to describe a method for expansion of human amniotic epithelial cells.

Another object of the present invention is to describe methods for cryopreservation of human amniotic epithelial cells.

Another object of the present invention is to describe methods for reculturing of frozen amniotic epithelial cells.

Another object of the present invention is to describe methods for assessing viability, proliferation potential, and longevity of human amniotic epithelial cells.

Another object of this invention is to describe methods for establishing a stable cell line(s) from primary human amniotic epithelial cells by exposure to selected chemical carcinogens.

Another object of this invention is to describe methods to induce differentiation of amniotic epithelial cells to cells of different lineage, as evidenced by changes in cellular antigens.

Another object of this invention is to describe therapeutic applications for these cells including, but not limited to:

a) Autologous/heterologous enzyme replacement therapy;

b) Autologous/heterologous transgene carriers in gene therapy;

c) Autologous/heterologous tissue regeneration/replacement therapy;

d) Reconstructive treatment by surgical implantation; and

e) Reconstructive treatment of tissues with products of these cells.

IV. Brief Description of the Drawings

Figure 1 is a phase-contrast image of amniotic epithelial cells shortly after isolation according to the procedure of Example 1.

Figure 2 is a phase-contrast image of amniotic epithelial cells isolated according to the procedure of Example 1 after attachment of the cells to a culture dish.

V. Detailed Description of the Preferred Embodiments of the Invention

In accordance with the present invention, human amniotic membranes or human amniotic epithelial cells are extracted from fresh human placenta.

According to a preferred embodiment of the invention, the placenta is harvested immediately after delivery, washed with sterile saline, and thereafter handled under sterile conditions. The amniotic membrane is then separated from the chorion. This separation may be accomplished by methods known to those of skill in the art. For example, the separation may be accomplished mechanically by using tweezers to lift the amnion from the chorion and then cutting the amniotic tissue in a circle around the umbilical cord.

The amniotic membrane itself may be cryopreserved in a cryoprotective solution comprising a medium or buffer and a cryoprotective agent. Examples of media are Dulbecco's Modified Eagle Medium (DMEM), Medium 199 (M199), F-12 Medium, and RPMI Medium. An example of a buffer is phosphate buffered saline (PBS). Examples of cryoprotective agents are dimethylsulfoxide (DMSO) and glycerol. Examples of cryoprotective solutions are: DMEM/glycerol (1:1), DMEM/7.5% DMSO, M199/7.5% DMSO, andPBS/3.5 M DMSO. Optionally, the amniotic membrane may be treated with antibiotics such as penicillin or streptomycin prior to cryopreservation. Cryopreservation may be accomplished using a rapid, flash-freeze method or by more conventional controlled rate-freeze methods. Rapid freezing of amniotic tissue may be accomplished by placing amniotic membrane sample(s) in a freezing tube containing a cryoprotective solution and then rapidly immersing the freezing tube in liquid nitrogen. General slow freezing may be accomplished by placing amniotic membrane sample(s) in a freezing tube containing a cryoprotective solution and then placing the freezing tube in a -70° C freezer. Alternatively, the amniotic epithelial sample(s) may be subjected to controlled rate freezing using a standard cryogenic rate controlled system.

In addition to cryopreserving the amniotic membrane itself, amniotic epithelial cells may be isolated from the amniotic membrane and then cryopreserved in a cyoprotectant solution such as the ones listed above. The amniotic tissue may be treated with antibiotics such as penicillin and/or streptomycin before or after digestion or both. The amniotic epithelial cells may be isolated from the amniotic membrane according to standard cell isolation techniques (15). For example, the amniotic membrane may be treated with trypsin/EDTA and/or collagenase and may be mechanically disrupted using plastic policemen. Also selective adhesion techniques may be used to eliminate mesenchymal fibroblasts. Additionally, the amniotic membrane may be treated with dispase, and the cells sheets may be treated with trypsin/EDTA.

Human amniotic epithelial cells are characterized by round, cobblestone morphology, large nuclei, epithelial membrane antigen and cytokeratin staining, and gap junctional communication.

The amniotic epithelial cells may be cultured in various media, such as DMEM, F-12, M199, RPMI and combinations thereof, supplemented with fetal bovine serum (FBS), whole human serum (WHS), or human umbilical cord serum collected at the time of delivery of the placenta from which the cells are extracted, or supplemented with growth factors, cytokines, hormones, vitamins, or any combination thereof. Alternatively, the amniotic epithelial cells may be cultured on feeder cells, such as irradiated fibroblasts, obtained from the same placenta as the amniotic epithelial cells or from other human or nonhuman sources, or in conditioned media obtained from cultures of such feeder cells, in order to obtain continued long-term culture of amniotic epithelial cells.

The amniotic epithelial cells may also be expanded in the presence of an agent which suppresses cellular differentiation. Such agents are well-

known in the art (16). Examples of agents which suppress cellular differentiation include leukemia inhibitory factor (LIF) and stem cell factor. On the other hand, agents such as hydrocortisone, Ca^{2+}, keratinocyte growth factor (KGF), TGF-β, retinoic acid, insulin, prolactin, sodium butyrate, TPA, DMSO, NMF, DMF, collagen, laminin, heparan SO_4, androgen, estrogen, and combinations thereof may be used to induce differentiation of these amniotic epithelial cells (15).

The amniotic epithelial tissue or cells may then be subjected to rapid, flash-freezing whereby the tissue/cells are placed in a freezing tube containing a cryoprotective solution and then the tube is rapidly immersed in liquid nitrogen.

On the other hand, the amniotic epithelial cells may be subjected to general controlled-rate (slow) freezing. General slow freezing may be accomplished by placing amniotic epithelial cells in a freezing tube containing a cryoprotective solution and then placing the freezing tube in a -70° C freezer. Alternatively, the amniotic epithelial cells can be subjected to controlled rate freezing using a standard cryogenic rate controlled system.

The amniotic membrane tissue that has been cryopreserved may be thawed and digested to obtain amniotic epithelial cells. These cells can then be cultured in the same manner as fresh amniotic epithelial cells for further use. In another embodiment of the invention, amniotic epithelial cells that have been cryopreserved are thawed and recultured for further use. Amniotic epithelial cells thus obtained from either thawed amniotic membranes or thawed cell cultures may be cultured in various media, such as DMEM, F-12, M199, RPMI, or combinations thereof, supplemented with fetal bovine serum, whole human serum, or matched human umbilical cord serum collected at the time of delivery of the placenta from which the cells ultimately derive. Alternatively, such amniotic epithelial cells may be treated with a prepared

mixture of growth factors, cytokines, hormones, and/or vitamins. Such amniotic epithelial cells may be assessed for viability, proliferation potential, and longevity using standard techniques in the art. For example, a trypan blue exclusion assay, a fluorescein diacetate uptake assay, a propidium iodide uptake assay, or other techniques known in the art may be used to assess viability. A thymidine uptake assay, an MTT cell proliferation assay, or other techniques known in the art may be used to assess proliferation. Longevity may be determined by the maximum number of population doublings in extended cultures or other techniques known in the art.

Additionally, amniotic epithelial cells derived from cryopreserved amniotic tissue or cells may be used to establish a stable cell line(s) by exposure to selected chemical carcinogens or by other techniques known in the art. Also, cells of different lineage(s) may be derived by inducing differentiation of amniotic epithelial cells as evidenced by changes in cellular antigens. Various epithelial differentiation-inducing agents are used to accomplish such differentiation, such as growth factors (for example EGF, aFGF, bFGF, PDGF, TGF-β), hormones (including but not limited to insulin, triiodothyronine, hydrocortisone, and dexamethasone), cytokines (for example IL-1α or β, IFN-γ, TFN), matrix elements (for example collagen, laminin, heparan sulfate, Matrigel), retinoic acid, transferrin, TPA, and DMSO. Such differentiation-inducing agents are known to those of ordinary skill in the art (15). Identification of differentiated cells may be accomplished by staining the cells with tissue-specific antibodies according to techniques known in the art.

The multipotentiality of the amniotic epithelial cells may also be demonstrated by their ability to form teratomas after injection into nude or severe combined immunodeficient (SCID)-beige mice, as has been demonstrated for human embryonic stem cells (2). However, in contrast to human embryonic stem cells whose use has raised ethical concerns, human

amniotic epithelial cells are derived from a readily available source (placenta) which is normally discarded after birth. Thus, cultured human amniotic epithelium is an ideal candidate for use in regenerative and/or reconstructive surgery, as well as for use in gene therapy. Some specific applications of human amniotic epithelial cells are described below.

Amniotic epithelial cells isolated from placenta and derived from cryopreserved amniotic tissue and/or cells may be used in autologous/heterologous enzyme replacement therapy in specific conditions including, but not limited to, lysosomal storage diseases, such as Tay-Sachs, Niemann-Pick, Fabry's, Gaucher's, Hunter's, Hurler's syndrome, as well as other gangliosidoses, mucopolysaccharidoses, and glycogenoses.

Additionally, amniotic epithelial cells isolated from placenta and derived from cryopreserved amniotic tissue and/or cells may be used as autologous/heterologous transgene carriers in gene therapy to correct inborn errors of metabolism affecting the cardiovascular, respiratory, gastrointestinal, reproductive, and nervous systems, or to treat cancer and other pathological conditions.

Amniotic epithelial cells isolated from placenta and derived from cryopreserved amniotic tissue and/or cells also may be used in autologous/heterologous tissue regeneration/replacement therapy, including but not limited to treatment of corneal epithelial defects, cartilage repair, facial dermabrasion, burn and wound dressing for traumatic injuries of skin, mucosal membranes, tympanic membranes, intestinal linings, and neurological structures.

Amniotic epithelial cells isolated from placenta and derived from cryopreserved amniotic tissue and/or cells also may be used in reconstructive treatment of damaged tissue by surgical implantation of cell sheets,

disaggregated cells, and cells embedded in carriers for regeneration of tissues for which differentiated cells have been produced.

Products of amniotic epithelial cells isolated from placenta and derived from cryopreserved amniotic tissue and/or cells also may be used in reconstructive treatment, either *in vivo* or *ex vivo*. Examples of such products include growth factors, cytokines, and other biological response modifiers.

The invention will be further clarified by the following examples, which are intended to be purely exemplary of the invention.

VI. Examples

Example 1

Immediately after delivery, a human placenta was harvested and washed with sterile saline. Under sterile conditions, the amnion was separated from the chorion using tweezers to lift the amnion from the chorion. The amnion was then cut in a circle around the umbilical cord, and then cut in wedged-shape pieces radiating outward from the cord. The membrane was immediately placed in a few hundred milliliters of sterile phosphate-buffered saline with100 IU/ml penicillin and 100ug/ml streptomycin where it was cut into small strips (approximately 2 x 2 cm) for immediate freezing or for further digestion to dissociate the cells.

Example 2

The strips of amniotic tissue prepared according to Example 1 were incubated in a few milliliters of 0.05% trypsin/ 0.53 mM EDTA for 0.5 hr at 37° C in a humidified CO_2 incubator. Then, the tissue was transferred to another culture dish containing a few milliliters of 0.03% collagenase in PBS and incubated for another 1.5 hrs in the humidified CO_2 incubator, with

periodic mechanical disruption of the tissue using plastic policemen. The disaggregated amniotic tissue was then pipetted and transferred to a conical tube for further incubation in approximately 10ml collagenase in a shaking water bath. The cells were then pelleted and resuspended in 10 ml M199 medium containing 10% fetal bovine serum, 2mM glutamine, and 100 IU/ml penicillin and 100 ug/ml streptomycin and placed in culture dishes. The cells were released from the culture for splitting using a combination of dispase at 2U/ml in medium and 0.05% trypsin/0.53 mM EDTA. Phase-contrast images of amniotic epithelial cells isolated in this manner are shown in Figures 1 and 2.

Example 3

For rapid freezing of tissue, whole pieces of amniotic tissue prepared according to Example 1 were sectioned and placed on sterile gauze with basal side up (away from gauze). The gauze was then lightly rolled and placed in a freezing tube containing approximately 10 ml of one of the following cryoprotective solutions: DMEM/glycerol (1:1), DMEM/7.5% DMSO, M199/7.5% DMSO, PBS/3.5 M DMSO. The tubes of amniotic tissue or cells were then rapidly immersed in liquid nitrogen, whereupon the samples froze within seconds.

Example 4

General slow freezing was accomplished by placing vials of amniotic tissue in cryoprotective solution prepared according to Example 1 or amniotic cells prepared according to Example 2 in a foam box in a –70° C freezer. Alternatively, disaggregated amniotic cells prepared according to Example 2 were suspended in 1 ml cryoprotective solution and subjected to rate freezing. Cryoprotectants included: DMEM/7.5% DMSO, M199/7.5% DMSO,

and DMEM/glycerol (1:1). Controlled rate freezing was accomplished using a Forma Scientific Cryomed Freezing Chamber, Model 8026 controlled by a Cryomed Model 1010 Programmable Freezing System. Cryomed's standard Program 1 was used for controlled rate freezing. All samples were kept for long-term storage in a Forma Scientific Liquid Nitrogen Storage System (Cryoplus 7400).

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification, examples, and claims be considered as exemplary only.

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WHAT IS CLAIMED IS:

1. A substantially purified human amniotic epithelial cell obtained from placentas at delivery, characterized by:

Round, cobblestone morphology;

Large nuclei;

Cytokeratin;

Epithelial membrane antigen; and

Gap junctional communication.

2. A method for obtaining substantially purified human amniotic epithelial cells comprising:

a) obtaining amniotic tissue from one or more human placenta, and

b) isolating human amniotic epithelial cells from the amniotic tissue.

3. The method of claim 2, further comprising eliminating mesenchymal fibroblasts.

4. The method of claim 3, wherein the mesenchymal fibroblasts are eliminated by procedures involving treatments with dispase and trypsin/EDTA, in addition to selective adhesion to plastic.

5. The method of claim 2, further comprising culturing the human amniotic epithelial cells in a culture media.

6. The method of claim 5 wherein the culture media is selected from the group consisting of DMEM, F-12, M199, RPMI, and combinations thereof.

7. The method of claim 5, wherein the culture media is supplemented with fetal bovine serum, whole human serum, or human

umbilical cord serum collected at the time of delivery of placenta from which the amniotic tissue is extracted.

8. The method of claim 5, wherein the culture media is supplemented with growth factors, cytokines, hormones, and/or vitamins.

9. The method of claim 5, wherein the human amniotic epithelial cells are cultured on feeder cells.

10. The method of claim 9, wherein the feeder cells are obtained from the same human placenta as the amniotic tissue.

11. The method of claim 9, wherein the feeder cells are obtained from nonhuman sources.

12. The method of claim 9, wherein the feeder cells are obtained from human sources.

13. The method of claim 9, wherein the feeder cells are irradiated fibroblasts.

14. The method of claim 9, wherein the culture media is supplemented with conditioned media obtained from cultures of fibroblasts obtained from the same human placenta as the amniotic tissue.

15. The method of claim 5, further comprising expanding the human amniotic epithelial cells in the presence of an agent which suppresses cellular differentiation.

16. The method of claim 15, wherein the agent which suppresses cellular differentiation is leukemia inhibitory factor or stem cell factor.

17. A method of cryopreserving human amniotic tissue, comprising:

a) obtaining amniotic tissue from one or more human placenta,

and

b) freezing the human amniotic tissue in a cryoprotective solution containing a cryoprotective agent.

18. The method of claim 17, wherein the freezing is flash-freezing.

19. The method of claim 18, wherein the flash-freezing is accomplished by immersing a container containing the human amniotic tissue in cryoprotective solution into liquid nitrogen.

20. The method of claim 17, wherein the freezing is controlled rate freezing.

21. The method of claim 20, wherein the cryoprotective agent is DMSO or glycerol.

22. The method of claim 17, further comprising thawing the frozen human amniotic tissue, and isolating human amniotic epithelial cells from the human amniotic tissue.

23. The method of claim 22 , further comprising culturing the human amniotic epithelial cells in a culture media.

24. The method of claim 23 wherein the culture media is selected from the group consisting of DMEM, F-12, M199, RPMI, and combinations thereof.

25. The method of claim 23, wherein the culture media is supplemented with fetal bovine serum, whole human serum, or human

umbilical cord serum collected at the time of delivery of placenta from which the amniotic tissue is extracted.

26. The method of claim 23, wherein the culture media is supplemented with growth factors, cytokines, hormones, and/or vitamins.

27. The method of claim 23, further comprising assessing the viability, proliferation potential, and/or longevity of the thawed human amniotic epithelial cells.

28. The method of claim 27, wherein the viability of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of trypan blue exclusion assay, fluorescein diacetate uptake assay, and propidium iodide uptake assay.

29. The method of claim 27, wherein the proliferation potential of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of thymidine uptake assay and MTT proliferation assay.

30. The method of claim 27, wherein the longevity of the thawed human amniotic epithelial cells is assessed by analyzing the number of population doublings in extended cultures.

31. A method of cryopreserving substantially purified human amniotic epithelial cells, comprising:

a) obtaining amniotic tissue from one or more human placenta,

b) isolating human amniotic epithelial cells from the amniotic tissue,

and

c) freezing the human amniotic epithelial cells in a cryoprotective solution containing a cryoprotective agent.

32. The method of claim 31, wherein the freezing is flash-freezing.

33. The method of claim 32, wherein the flash-freezing is accomplished by immersing a container containing the human amniotic epithelial cells in cryoprotective solution into liquid nitrogen.

34. The method of claim 31, wherein the freezing is controlled rate freezing.

35. The method of claim 34, wherein the cryoprotective agent is DMSO or glycerol.

36. The method of claim 31, further comprising thawing the frozen human amniotic epithelial cells, and reculturing the human amniotic epithelial cells in a culture media.

37. The method of claim 36 wherein the culture media is selected from the group consisting of DMEM, F-12, M199, RPMI, and combinations thereof.

38. The method of claim 36, wherein the culture media is supplemented with fetal bovine serum, whole human serum, or human umbilical cord serum collected at the time of delivery of placenta from which the amniotic tissue is extracted.

39. The method of claim 36, wherein the culture media is supplemented with growth factors, cytokines, hormones, and/or vitamins.

40. The method of claim 36, further comprising assessing the viability, proliferation potential, and/or longevity of the thawed human amniotic epithelial cells.

41. The method of claim 40, wherein the viability of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of trypan blue exclusion assay, fluorescein diacetate uptake assay, and propidium iodide uptake assay.

42. The method of claim 40, wherein the proliferation potential of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of thymidine uptake assay and MTT proliferation assay.

43. The method of claim 40, wherein the longevity of the thawed human amniotic epithelial cells is assessed by analyzing the number of population doublings in extended cultures.

44. The method of claim 2, further comprising establishing a stable cell line(s) from the human amniotic epithelial cells by exposure to selected chemical carcinogens.

45. The method of claim 44, further comprising continued culture of the stable cell line(s) derived from the human amniotic epithelial cells.

46. The method of claim 44, further comprising cryopreserving the stable cell line(s) from the human amniotic epithelial cells.

47. The method of claim 46, further comprising thawing the frozen stable cell line(s) from the human amniotic epithelial cells, and reculturing the stable cell line(s) from the human amniotic epithelial cells in a culture media.

48. The method of claim 46, further comprising assessing the viability, proliferation potential, and/or longevity of the thawed stable cell line(s) from the human amniotic epithelial cells.

49. The method of claim 48, wherein the viability of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of trypan blue exclusion assay, fluorescein diacetate uptake assay, and propidium iodide uptake assay.

50. The method of claim 48, wherein the proliferation potential of the thawed human amniotic epithelial cells is assessed using a test selected from the group consisting of thymidine uptake assay and MTT proliferation assay.

51. The method of claim 48, wherein the longevity of the thawed human amniotic epithelial cells is assessed by analyzing the number of population doublings in extended cultures.

52. The method of claim 44, further comprising inducing differentiation of human amniotic epithelial cells by exposing the stable cell line(s) from the human amniotic epithelial cells to one or more epithelial differentiation-inducing agents.

53. The method of claim 52, wherein the epithelial differentiation-inducing agents are selected from the group consisting of growth factors, EGF, aFGF, bFGF, PDGF, TGF-β, hormones, insulin, triiodothyronine, hydrocortisone, dexamethasone, steroids, cytokines, IL-1α, IL-1β, IFN-γ, TNF, matrix elements, collagen, laminin, heparan sulfate, Matrigel, retinoic acid, transferrin, TPA, and DMSO.

54. The method of claim 52, further comprising evaluating the differentiation of the human amniotic epithelial cells by staining the human amniotic epithelial cells with tissue-specific antibodies.

55. A method of treating a lysosomal storage disease comprising administering to a patient suffering therefrom substantially purified human amniotic epithelial cells.

56. The method of claim 55 wherein said lysosomal storage disease is Tay-Sachs disease, Niemann-Pick disease, Fabry's disease, Gaucher's disease, Hunter's disease, Hurler's disease, a gangliosidosis, a mucopolysaccharidosis, or a glycogenosis.

57. A method of correcting inborn errors of metabolism affecting cardiovascular, respiratory, gastrointestinal, reproductive, or nervous systems, comprising implanting differentiated cells derived from purified human amniotic epithelial cells.

58. The method of claim 57 wherein the purified human amniotic epithelial cells have been transfected with specific gene(s).

59. A method of treating cancer in a patient suffering from cancer comprising using substantially purified human amniotic epithelial cells as autologous or heterologous transgene carriers in gene therapy.

60. A method of treating corneal epithelial defects, cartilage damage, or facial dermabrasion in a patient suffering from said corneal epithelial defects, cartilage damage, or facial dermabrasion, comprising replacing damaged tissue with substantially purified human amniotic epithelial cells and/or causing said damaged tissue to be regenerated using substantially purified human amniotic epithelial cells.

61. A method of treating corneal epithelial defects, cartilage damage, or facial dermabrasion in a patient suffering from said corneal epithelial defects, cartilage damage, or facial dermabrasion, comprising using substantially purified human amniotic epithelial cells as a burn or wound dressing.

62. A method of reconstructive treatment of damaged tissue comprising surgically implanting substantially purified human amniotic epithelial cells in the form of cell sheets, disaggregated cells, and cells embedded in carriers for regeneration of tissues for which differentiated cells have been produced.

63. A method of reconstructive treatment of tissues in patients comprising administering growth factors, cytokines, and other biological response modulators derived from substantially purified human amniotic epithelial cells.

64. The method of claim 63, wherein the treatment is *in vivo*.

65. The method of claim 63, wherein the treatment is *ex vivo*.

66. A substantially purified human amniotic epithelial cell prepared according to the method of claim 2.

67. A substantially purified human amniotic epithelial cell prepared according to the method of claim 31.

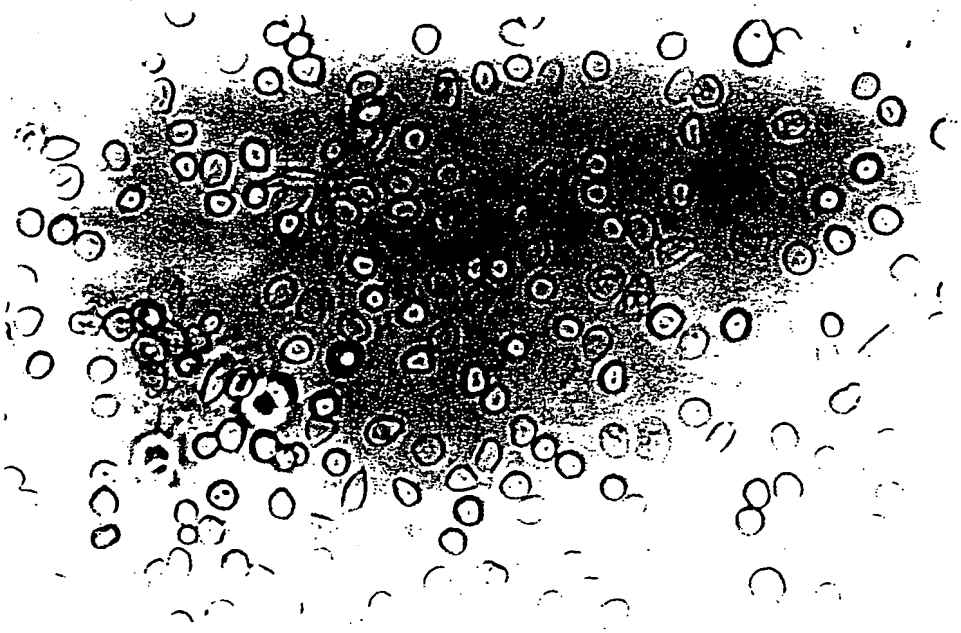

Fig. 1. Human amniotic epithelial cells just after isolation from amnion.

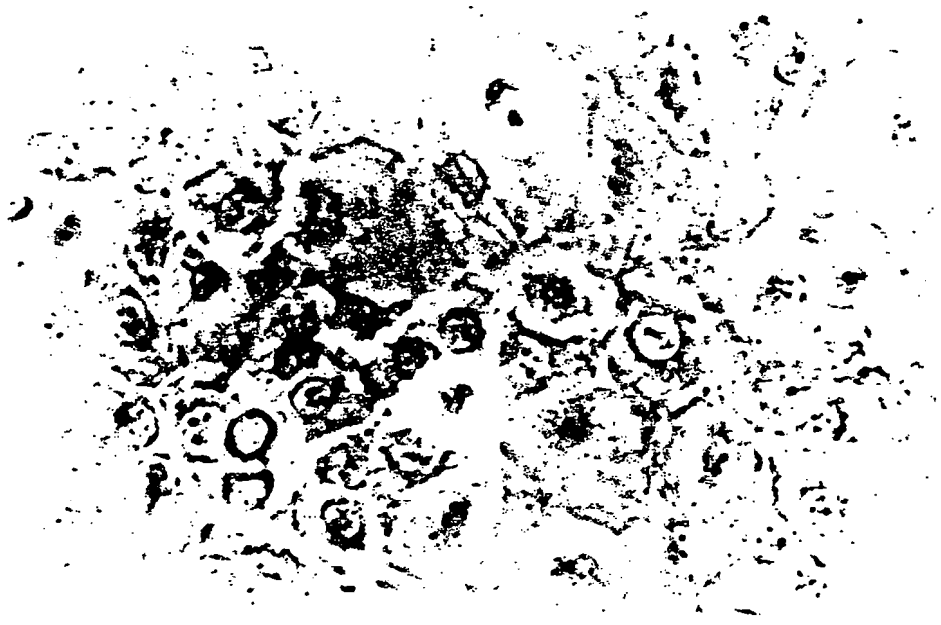

Fig. 2. Human amniotic epithelial after a week in culture.

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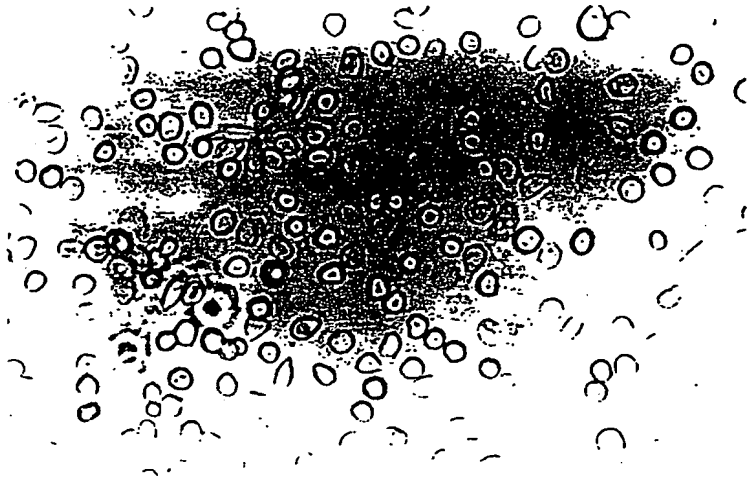
(54) Title: METHODS OF ISOLATION, CRYOPRESERVATION, AND THERAPEUTIC USE OF HUMAN AMNIOTIC EPITHELIAL CELLS



Human amniotic epithelial cells just after isolation from amnion.

(57) Abstract: The present invention is directed to human amniotic epithelial cells derived from placenta at delivery, and the methods for isolating, culturing, and cryopreserving them for future therapeutic uses. Additionally, the present invention is directed to methods for inducing differentiation of these multipotentials cells, for assaying the cell types derived, and for manipulating the cells by gene transfection and other means for therapeutic applications, icluding but not limited to enzyme replacement and gene therapy, tissue regeneration and replacement, as well as burn and wound dressings.

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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EPO-Internal, WPI Data, PAJ, BIOSIS

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